REMARKS

Applicants acknowledge receipt of the Office Action dated August 8, 2006.

Status of Claims:

Claims 1-9 and 18-19 are withdrawn;

Claim 14 has been canceled;

Claims 10, 15 and 20 are currently amended;

New claim 23 has been added; and

Claims 10–13, 15–17, and 20–23 are pending in the application.

Amendment to the Specification.

In the Office Action it was noted that the specification contains a large blank area on page 17, and it was said that it was unclear whether the remaining area on page 17 after lines 1–4 was left blank intentionally or whether some content is missing. Appropriate correction was required. In reply, Applicants have requested deletion of the blank area in question. As corrected, and as originally intended, the next line of text immediately following line 4 of page 17 now begins with "Table 1 – RNA apatamer tags tested for use in TRAP vectors," which is the first line of page 18.

Claim Amendments.

Claim 10 is currently amended to incorporate the feature of at least one insulator sequence. Basis for this feature can be found at page 3, lines 12-13, of the PCT application as filed, which states as follows: "In another embodiment of the current invention, the RNA fusion molecules further comprise at least one insulator sequence."

Claim 12 is amended to remove multiple dependency, and now depends from only claim 10. In conjunction with this amendment, new claim 23 is added to recite the deleted subject matter of claim 12 (*i.e.*, it depends from claim 11).

Claims 15 and 20 are currently amended to remove dependency from withdrawn or canceled claims.

Objections to Claims.

Claims 14, 15 and 20 are objected to in the Office Action as containing non-elected subject matter. As indicated above, claim 14 is now canceled, and claims 15 and 20 are currently amended to delete dependency from claim 9 and claim 14.

Claims 14, 15 and 20 are also objected to as being in improper multiple dependent form. Applicants respectfully submit that the wording of claims 15 and 20, as amended, fully complies with MPEP § 608.01(n).

Claim Rejections under 35 U.S.C. § 102(a)

In the Office Action, claims 10, 12, 15–17 and 20–22 are rejected as being anticipated by *Srisawat et al.* It is said that all of the limitations of these claims are met by *Srisawat et al.* As noted above, Applicants have amended claim 10 to incorporate the limitation "at least one insulator sequence" which was originally recited in claim 14. In the Office Action, claim 14 (now canceled) was not considered to be anticipated by *Srisawat et al.*

Srisawa et al. do not disclose *all* of the essential elements of the current invention, such as, the insulator elements. This single reference may provide some vague guidelines for tagging RNA, however, those general principles are not absolutes and they are not necessarily and inevitably applicable in each and every instance. Although Applicants state in the Specification (at page 15, lines 29–30) that "[i]nsulator elements may also be called spacers," it is nevertheless clear that the spacer elements of *Srisawat* are not the same as the insulator or spacer sequences of the current invention. The insulator sequences of the current invention are multiple palindromic restriction sites which "function to ensure proper folding of the tags and to discourage interactions between the tags and the inserted target RNA" (page 15, lines 22–24 of the Specification). By contrast, the short, non-palindromic spacers suggested in *Srisawat* are not workable for this purpose. Indeed the spacers disclosed in *Srisawat* are likely to interfere with the folding of the tags or the target RNA. Applicants, therefore, respectfully submit that claims 10, 12, 15–17 and 20–22, as currently amended, are novel over the cited reference.

Claim Rejections under 35 U.S.C. § 103(a) - *Srisawat et al.* and *Rigaut et al.*

Claims 10–12, 14–17 and 20–22 are rejected in the Office Action as being unpatentable over *Srisawat et al.*, as applied above to claims 10, 12, 15–17 and 20–22, in view of *Rigaut et al.* In reply, Applicants respectfully traverse this rejection and submit that, although it may have been considered worthwhile to explore the path of tandem tags that would specifically increase the purity of an RNA-protein complex, it was not obvious to one of ordinary skill in the art at the time of the present invention. The way to achieve such a level of purity was not known at that time. A person of ordinary skill in the art, in view of the cited references and the common

general knowledge at the relevant time, could not have known how to achieve the specific constructs of the current invention. Indeed, *Srisawat et al.* state that there is a high level of unpredictability in the relevant field of art and that "it is often necessary to generate and test several tagged RNA constructs to ensure that they have normal biological functions and are still able to bind to the affinity matrix" (pg. 158, col. 2, line 23 – pg. 159, col. 1, line 1 of *Srisawat et al.*). Inventive ingenuity was required to achieve the desired level of purity attained by the constructs and methods of the current invention.

The examples of the instant application detail the nature of the insulator sequences for use in the disclosed methods, and provide instructions for the construction of TRAP-tag vectors. From the data provided, it is clear that the TRAP-tags of the instant invention produce functional complexes between RNA and protein isolated from cellular extracts. Applicants submit that, although multiple single tags were known, the use of multiple TAP tags or RNA tags would not have been obvious to a person of ordinary skill in the art at the time of Applicants' invention.

In order to routinely identify components of RNP complexes by mass spectrometry/microarrays, Applicants deduced that purification factors exceeding 1000-fold, with high yields, would be necessary, especially when starting with complex mixtures such as tissue or whole animal lysates. This would not have been obvious to the artisan. The need for these levels of purification for any type of protein complex component identification in higher eukaryotes is first discussed in Yang *et al.* (Proteomics, 2006). Applicants demonstrated that even the TAP tag is insufficient for this purpose.

In the *Srisawat et al.* construct, only one of the two tags worked. This outcome emphasizes the importance of Applicants' invention, which provides tag selection and tag orientation, placement and separation, which allow both tags to function. Many different tags and tag orientations do not work. Tag choice, number, spacing and orientation are crucial.

In order to perform sequential purification steps, tags chosen must bind with sufficient affinity to the column matrix to isolate the majority of tagged RNA/RNP complexes from cell and tissue isolates, and must then be efficiently released without disrupting RNA/RNP complexes. Only a few work sufficiently well to be useful.

Regarding claims 12, 13 and new claim 23, the second tag used by Applicants, MS2/coat protein, had not previously been used for affinity purification purposes. It would not have been

obvious to a person skilled in the art that this tag would work in this context and, if it did, that it would work sufficiently well. This is also true for any other potential tag/ligand system.

The chosen tags and ligands must be readily available or synthesized to be useful. For example, streptavidin beads are readily available in a form suitable for this purpose. Applicants, however, applied ingenuity in creating an MS2 protein expression purification procedure that yielded soluble and functional protein. It was also necessary that Applicants developed a coupling procedure that yielded a useful matrix in which the protein was still active and did not leach off the column during purification.

The chosen tags and ligands must also be highly specific. Many ligands have fairly high non-specific RNA binding affinity, which leads to unacceptably high background. Eluates and buffers used to remove complexes from columns must not interfere with mass spectrometry. This is the case, for example, if coat protein or streptavidin are in the final eluate. Applicants' invention circumvents this issue. Applicants, therefore, respectfully submit that claims 10–12, 14–17 and 20–22, as currently amended, are non-obvious over the combined references.

Claim Rejections under 35 U.S.C. § 103(a) - *Srisawat et al.* and *Johansson et al.*

In the Office Action, claims 10, 12–13, 15–17 and 20–22 are rejected under 35 U.S.C. § 103(a) as being unpatentable over *Srisawat et al.* in view of *Johansson et al.* It is said that it would have been obvious to one of ordinary skill in the art at the time of the instantly claimed invention to make an RNA fusion molecule having at least one MS2 coat protein binding sequence of *Johansson et al.* by modifying the teachings of *Srisawat et al.*, which teach an RNA fusion molecule comprising an RNA target sequence and S1 and D8 RNA tags. As discussed above, Applicants have included the limitation of claim 14, that is, the feature of at least one insulator element, into independent claim 10. Claim 14, now canceled as being duplicative of claim 10, as amended, is not rejected over these references. Since neither *Srisawat et. al.* nor *Johansson et al.* teach or suggest the insulator sequence, as defined by Applicants, in an RNA fusion molecule, it is respectfully submitted that claims Applicants, therefore, respectfully submit that claims 10, 12–13, 15–17 and 20–22 , as currently amended, are non-obvious over *Srisawat et al.* in view of *Johansson et al.*

No new matter is added by this amendment. Accordingly, Applicants believes these amendments address the Examiner's concerns and respectfully request withdrawal of the objection.

Conclusion

Applicants respectfully request reconsideration and withdrawal of the objections and rejections, and allowance of the pending claims. If the Examiner feels that a telephone conference would expedite the resolution of this case, the Examiner is invited to contact the undersigned.

In the course of the foregoing discussions, Applicants may have at times referred to claim limitations in shorthand fashion, or may have focused on a particular claim element. This discussion should not be interpreted to mean that the other limitations can be ignored or dismissed. The claims must be viewed as a whole, and each limitation of the claims must be considered when determining the patentability of the claims. Moreover, it should be understood that there may be other distinctions between the claims and the prior art that have yet to be raised, but which may be raised in the future.

It is believed that no extensions of time or fees are required beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that any additional extension of time is necessary to allow consideration of this paper, such extension is hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required (including fees for net addition of claims) are hereby authorized to be charged to Deposit Account No. 03-2769 (ref. 1889-00900) of Conley Rose, P.C., Houston, Texas.

Respectfully submitted,

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